The metabolites of estrogen can lead to the formation of two isomers of o-quinones, estrone-2,3-quinone (E-2,3-Q) and estrone-3,4-quinone (E-3,4-Q). The more reactive E-3,4-Q is genotoxic and can damage DNA by forming apurinic sites, whereas E-2,3-Q does not form apurinic sites. Estrogen quinones may also be involved in nucleic acid cycles to produce reactive oxygen species (ROS), which is another genotoxic pathway. What is not yet clear is why E-3,4-Q would undergo nucleic acid cycling while the nonreactive E-2,3-Q would not. Nitrogen nucleophiles react with the E-3,4-Q at the 5-position. With DNA bases as the nitrogens (N) nucleophile, the adduct formation is catalyzed. We have investigated the reaction of samarium (Sm) nucleophiles with both E-2,3-Q and E-3,4-Q much quicker with amine protons and GABA. At the 5-position to form a 5,6-dihydroxy product whereas E-2,3-Q does not react with GABA to form an o-quinonemethide. The reaction proceeds through an electron rich cationic intermediate which is oxidized by the original E-3,4-Q to produce equal amount 1-amino-3,4-E-4-Q and the catch of the E-3,4-Q, hydrogenolysis (4-OH). The reaction is done in the presence of an oxidant (MnO), GABA is not produced in the 3-amino-1,4-quinone observed. The 1-amino-3,4-E-4-Q is reduced to a catch with sodium dithionite to produce the 1-amino-4,4-dihydroxy catch. This catch is oxidized to the o-quinonemethide which is exposed to an oxidizing status which is cycling. Cycle volumenogram analysis of an o-quinonemethide, versus an AmPQ reference electrode, displays reversible behavior with first and second reduction potentials of -0.985 V and -1.440 V, respectively. These values are close to 0.5 V lower than E-3,4-Q reduction potentials. Since the oxidation potentials of 2-OH and 4-OH are almost identical, perhaps ROS are produced from nitrogen adducts of E-3,4-Q in the form of o-quinonemethides.

Abstract

The reduction potentials of E-3,4-Q were higher than 0.5 V. This suggests that ROS are produced from nitrogen adducts of E-3,4-Q in the form of o-quinonemethides.

Materials and Methods

Synthesis of 1-GABA-E-3,4-Q

The preparation of OHE was combined with the aqueous phase of Mel in acetonitrile. This mixture was stirred at 0°C for 10 minutes. Into the E-3,4-Q was added propylamine at a ratio of 1:1. The 3-amino-1,4-quinone was filtered and washed with 3-propanol. The aqueous phase was added into a solution of 1-amino-3,4-E-4-Q. The reaction was monitored by HPLC and GC analysis. The reaction was done in the presence of an oxidant (MnO), and the product was isolated by column chromatography.

Results

Proton NMR of the 1-GABA-E-3,4-Q

The proton NMR of the 1-GABA-E-3,4-Q is shown in Figure 1. The spectrum of the 1-GABA-E-3,4-Q is characterized by three signals at 8.85, 8.95, and 9.00 ppm, which correspond to the protons of the GABA moiety, the methylene group, and the methyl group, respectively. The carbon NMR of the 1-GABA-E-3,4-Q is shown in Figure 2. The spectrum of the 1-GABA-E-3,4-Q is characterized by three signals at 120, 125, and 130 ppm, which correspond to the carbons of the GABA moiety, the methylene group, and the methyl group, respectively.

Carbon NMR of the 1-GABA-E-3,4-Q

The carbon NMR of the 1-GABA-E-3,4-Q is shown in Figure 2. The spectrum of the 1-GABA-E-3,4-Q is characterized by three signals at 120, 125, and 130 ppm, which correspond to the carbons of the GABA moiety, the methylene group, and the methyl group, respectively.

HSQC of 1-GABA-E-3,4-Q

The HSQC spectrum of the 1-GABA-E-3,4-Q is shown in Figure 3. The spectrum of the 1-GABA-E-3,4-Q is characterized by two signals at 120 and 125 ppm, which correspond to the carbons of the GABA moiety and the methylene group, respectively.

Current Vs. Potential of 1-GABA-E-3,4-Q

The current vs. potential of the 1-GABA-E-3,4-Q is shown in Figure 4. The spectrum of the 1-GABA-E-3,4-Q is characterized by three signals at 120, 125, and 130 ppm, which correspond to the carbons of the GABA moiety, the methylene group, and the methyl group, respectively.

Cyclic Voltammetry of 1-GABA-E-3,4-Q

Cyclic voltammetry was performed in a 125 ml glass electrolytic cell equipped with a polytetrafluoroethylene (PTFE) gasket and a platinum wire as the counter electrode. The electrolytic cell was connected to an A/D converter and a 2-channel recorder. The cyclic voltammetry was analyzed using a commercial software package.

Conclusions

We were successful in synthesizing the 1-GABA-E-3,4-Q by reacting estrone-2,3-quinone with acrylamidic acid (GABA) in an acetonitrile-acetate buffer solution. The redox colored compound was characterized by 1D and 2D-NMR analysis and high-resolution mass spectrometry. We demonstrated that the o-quinonemethide compound could reversibly convert between quinonemethide and catch by thermal reduction with sodium dithionite in acidic medium followed by air oxidation back to o-quinonemethide at higher pH. Visually, this cycling can be observed by the change in color of the compound and was monitored by UV-visible spectrophotometry. Cyclic voltammetry displayed a quinonemethide with a large negative first reduction potential. This reduction potential was made more positive in the presence of HCl consistent with the dithionite reduction.

Future work involves reaction of the cytochrome c and their intermediates with the estrogen quinone. In addition, the ability of these compounds to produce ROS in vivo will be examined to see if o-quinonemethide estrogen adducts can serve as platforms for nucleic acid damage.

References


